Recombinant Lipoprotein lipase (LPL)

L1493004

Storage temperature: -20℃.

Introduction

Lipoprotein lipase is a multifunctional enzyme from adipose tissue, heart and skeletal muscle, islets and macrophages. Lipoprotein lipase promotes normal lipoprotein metabolism, delivery and utilization of tissue-specific substrates. Lipoprotein lipase catalyzes the rate-limiting step of lipids in blood circulation.

PREPARATION and SPECIFICATION

Appearance: Light brown amorphous powder, lyophilized

Activity: ≥20U/mg enzyme powder (containing approx. 80% of stabilizers)

Contaminants:

Phosphatase: ≤ 1.0×10^{-3}%

Catalase: ≤ 2.0×10^{-2}%

NADH oxidase: ≤ 1.0×10^{-2}%

Cholesterol oxidase: ≤ 2.0×10^{-2}%

Stabilizers: Mg^{2+}, Na-cholate, BSA

PROPERTIES

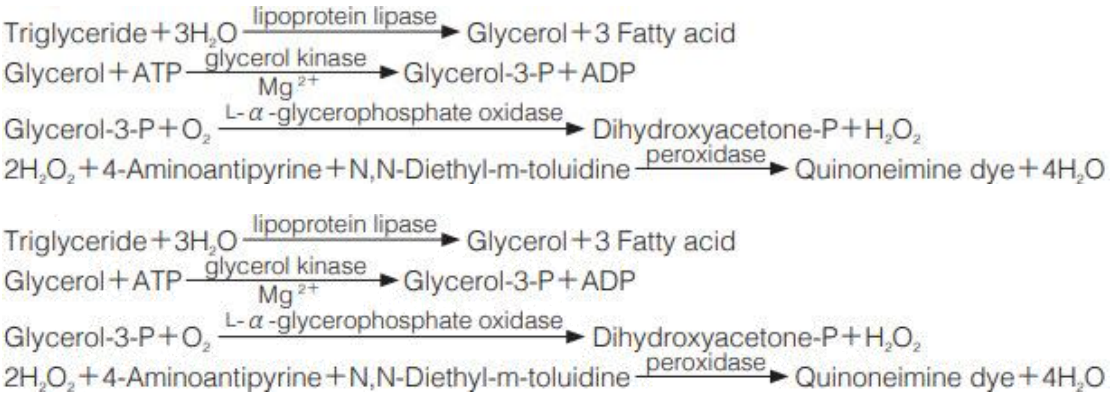
Stability	Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	approx. 134,000	
Isoelectric point	5.95±0.05	
Inhibitors	Hg^{2+}, Ag^{+}, ionic detergents	
Optimum pH	7.0−9.0	(Fig.4)
Optimum temperature	45−50℃	(Fig.5)
pH Stability	pH 7.0−9.0 (25℃, 20hr)	(Fig.6)
Thermal stability	below 55℃ (pH 7.0, 10min)	(Fig.7)
Substrate specificity	(Table 1)	
Effect of various chemicals	(Table 2)	

APPLICATIONS

This enzyme is useful for enzymatic determination of triglyceride in serum when in combination L-α-glycerophosphate oxidase andglycerol kinase. Usually, the reaction can be completed in 5 minutes at 37 ℃ by using 2.5~3.0 units of the enzyme per test (3.0 mL) at a pH

of around 7.0.

ASSAY Principle



Triglyceride + 3H2O
lipoprotein lipase
Glycerol + 3 Fatty acid
Glycerol + ATP
glycerol kinase
Mg2+
Glycerol-3-P + ADP
Glycerol-3-P + O2
L-α-glycerophosphate oxidase
Dihydroxyacetone-P + H2O2
2H2O2 + 4-Aminoantipyrine + N,N-Diethyl-m-toluidine
peroxidase
Quinoneimine dye + 4H2O

The formation of quinoneimine dye is measured at 545 nm by spectrophotometry.

Unit definition

One unit causes the formation of one micromole of glycerol (half a micromole of quinoneimine dye) per minute under the conditions detailed below.

Method

Reagents:

A. Olive oil emulsion Sonicate the mixture of 5.0 g of olive oil［reagent grade (highly refined, low acidity)］and 5.0 mL of 5.0 % Triton X-100 solution (B) for 10 minutes (20 KHz). To the oil emulsion, add 25 mL of 4.0 % BSA solution (C) and 15 mL of 0.1 M potassium phosphate buffer, pH 7.0 (D), and mix. (should be freshly prepared)

B. Triton X-100 solution 5.0 % (5.0 mL Triton X-100/100 mL of H_2O)

C. BSA solution 4.0 %［4.0 g bovine serum albumin/100 mL of H_2O］

D. K-phosphate buffer, pH 7.0 0.1 M

E. TCA solution 0.2 M (33 g trichloroacetic acid/1,000 mL of H_2O)

F. MES-NaOH buffer 50 mM MES buffer, pH 6.5: Dissolve 9.76 g of 2-(N-morpholino)-ethanesulfonic acid (MW ＝ 195.23) in approx. 850 mL of H_2O, and, after adjusting the pH to 6.5 with 5.0 N NaOH, make up to 1,000 mL with H_2O.

G. Color developing reagent Dissolve the following chemicals and enzymes in 200 mL of 50 mM MES buffer (F) in the following order:

4.0 mL	Triton X-100 solution (B)
0.04 mL	N,N-diethyl-m-toluidine (Stir until completely dissolved)
4.0 mg	4-Aminoantipyrine
24.2 mg	ATP·Na_2·$3H_2O$
40.7 mg	$MgCl_2$·$6H_2O$
200 units	Glycerol kinase

500 units	L-α-glycerophosphate oxidase
300 units	Peroxidase (purpurogallin units)

(Stable for one week if stored at 4 ℃ in a brownish bottle)

H. Enzyme diluent 20 mM Potassium phosphate buffer, pH 7.5, containing 2.0 mM $MgCl_2$ and 0.5 mM EDTA-Na3

Procedure:

(1st step)

1. Pipette 2.0 mL of olive oil emulsion (A) into a test tube and equilibrate at 37 ℃ for approximately 5 minutes.
2. Add 0.2 mLl of the enzyme solution* and mix.
3. After exactly 15 minutes at 37 ℃, add 2.0 mL of TCA solution (E) to stop the reaction and remove the precipitate by filtration with filter paper.

Concentration in assay mixture:

Potassium phosphate buffer	29.1 mM
Olive oil	90.9 mg/mL
$MgCl_2$	0.18 mM
Triton X-100	9.1 %
EDTA	45 μM
BSA	1.8 %

(2nd step)

4. Pipette 0.05 mL of the filtrate thus obtained into a test tube.
5. Add 3.0 mL of color developing reagent (G) and incubate at 37 ℃ for 15 minutes.
6. Measure the optical density at 545 nm against water (OD test).

At the same time, prepare the blank by mixing 2.0 mL of olive oil emulsion (A), after incubation for 10 minutes at 37 ℃, with 2.0 mL of TCA solution, followed by addition of the enzyme solution (1st step). Using the filtrate obtained from the mixture, carry out the 2nd step, using the same procedure as in the test, and measure the optical density at 545 nm (OD blank).

*Dissolve the enzyme preparation in ice-cold enzyme diluent (H) and dilute to 0.4−1.2 U/mL with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ test - OD\ blank) \times Vt\text{-}1 \times Vt\text{-}2 \times df}{28.2 \times 1/2 \times 1.0 \times t \times Vs\text{-}1 \times Vs\text{-}2} = \Delta OD \times 6.057 \times df$$

$$\text{Weight activity (U/mg)} = (U/ml) \times 1/C$$

Vt-1	Total volume in 1st step (4.2 mL)
Vt-2	Total volume in 2nd step (3.05 mL)
Vs-1	Sample volume in 1st step (0.2 mL)

Vs-2	Sample volume in 2nd step (0.05 mL)
28.2	Millimolar extinction coefficient of quinoneimine dye under the assay condition (cm²/micromole)
1/2	Factor based on the fact that one mole of H_2O_2 produces half a mole of quinoneimine dye
1.0	Light path length (cm)
t	Reaction time in 1st step (15 minutes)
df	Dilution factor
C	Enzyme concentration in dissolution (c mg/mL)

Table 1. Substrate Specificity of Lipoprotein lipase (Substrate:10 %)

Substrate	Relative activity(%)	Substrate	Relative activity(%)
Olive oil	94	Tricaprylin (8:0)	64
Triolein (18:1)	100	Tricaproin (6:0)	2
Tripalmitin (16:0)	2	Tributyrin (4:0)	2
Trimyristin (14:0)	7	Tripropionin (3:0)	2
Trilaurin (12:0)	4	Triacetin (2:0)	1
Tricaprin (10:0)	17		

Number of carbon atoms to number of double bonds is given in parenthesis.

Table 2. Effect of Various Chemicals on Lipoprotein lipase.

[The enzyme (2.5U/ml) was incubated at 25℃ for 1hr with each chemical.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	PCMB	2.0	100
$CaCl_2$	2.0	95	MIA	2.0	98
$Ba(OAc)_2$	2.0	92	NaF	20.0	95
$FeCl_3$	2.0	80	NaN_3	20.0	97
$CoCl_2$	2.0	90	EDTA	5.0	100
$MnCl_2$	2.0	85	o-Phenanthroline	2.0	100
$Zn(OAc)_2$	2.0	86	α,α'-Dipyridyl	2.0	94
$NiCl_2$	2.0	97	Borate	20.0	100
$Pb(OAc)_2$	2.0	80	Triton X-100	1.0%	100
$AgNO_3$	2.0	47	Brij 35	1.0%	100
$HgCl_2$	2.0	5	SDS	0.1%	4
$CdCl_2$	1.0	82	Tween 20	0.1%	89
$CrCl_2$	1.0	42	Span 20	0.1%	100
$SnCl_2$	1.0	49	Na-cholate	1.0%	93
$CuSO_4$	1.0	58	Taurocholate	0.1%	100

NEM	2.0	98			

Ac, CH_3CO; NEM, N-Ethylmaleimide; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; EDTA, Ethylenediamineteraacetate; SDS, Sodium dodecyl sulfate.

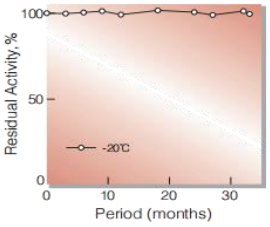


Fig.1. Stability (Powder form)
〔kept under dry conditions〕

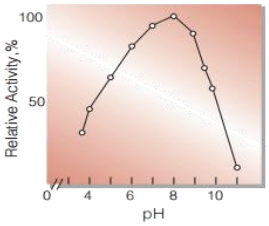


Fig.4. pH-Activity
〔37℃,in 0.1M Britton-Robinson buffer〕

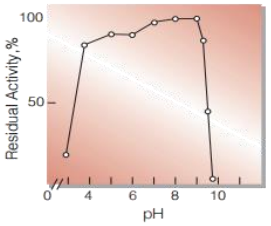


Fig.6. pH-Stability
〔25℃,20hr-treatment with 0.1M Britton-Robinson buffer〕

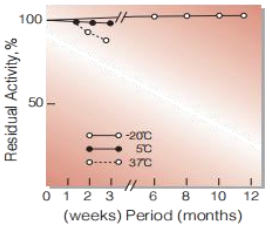


Fig.2. Stability (Powder form)
〔kept under dry conditions〕

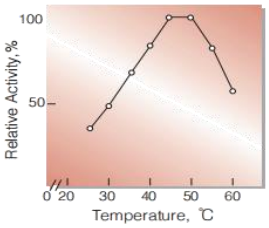


Fig.5. Temperature activity
〔in 0.1M K-phosphate buffer, pH7.0〕

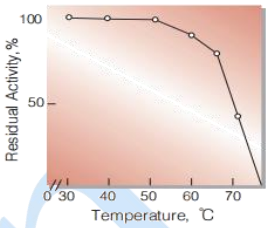


Fig.7. Thermal stability
〔10min-treatment with 0.1M K-phosphate buffer, pH7.0〕

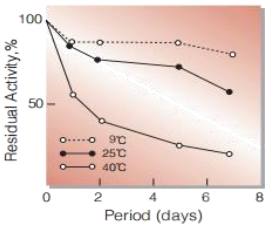


Fig.3. Stability (Liquid form)
〔in 20mM K-phosphate buffer,pH7.5 (contg. 2.0mM $MgCl_2$, 0.5mM EDTA.Na_2, 0.005% NaN_3) enzyme concn.:4U/ml〕